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NMR Spectroscopic Investigation of Inclusion Complexes between Cyclodextrins and the Neurotoxin Tetramethylenedisulfotetramine (TETS)

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Abstract

The binding stoichiometry, strength and structure of inclusion complexes formed between the neurotoxin tetramethylenedisulfotetramine (TETS) and both native and modified cyclodextrins (CyDs) were investigated using nuclear magnetic resonance (NMR) spectroscopy. Of all six examined cases, native β-cyclodextrin (β-CyD) and its chemically modified counterpart heptakis-(2,3,6-tris-(2-hydroxypropyl))-β-cyclodextrin (2HP-β-CyD) were found to associate most strongly with TETS as reflected in the magnitude of their binding constants ($K = 537 \pm 26$ M^{-1} for β-CyD and $K = 514 \pm 49$ M^{-1} for 2HP-β-CyD). Two-dimensional rotating-frame Overhauser effect spectroscopy (2D-ROESY) NMR experiments confirm close proximity of the TETS molecule to both β-CyD and 2HP-β-CyD as intermolecular, through-space interactions between the H_3 and H_5 protons located in the interior of the CyD cavity and the methylene protons of TETS were identified.

Keywords : Cyclodextrins, TETS, inclusion complex, NMR, Job plot, ROESY.

1. Introduction

Tetramethylenedisulfotetramine (Fig. 1), commonly known as TETS [1] is a caged, non-polar, lethal neurotoxin that has found successful utility as a rodenticide in China [2]. It is well established that TETS directly affects the nervous system by effectively blocking the γ-aminobutyric acid (GABA)-mediated chloride channel via noncompetitive and irreversible receptor binding [3-5]. The efficiency and potency of the blocking event is such that incredibly small amounts of TETS (oral LD_{50}: 0.1 mg kg^{-1}) in mammals can lead to convulsions and eventually death if not treated immediately, with an established lethal range of 7-10 mg kg^{-1} dosage for humans [6,7]. It has been estimated that there have been thousands of TETS poisonings through food in China, resulting in hundreds of human deaths from 1977-2002 [8,9], whereas there has been only one reported case of TETS poisoning within the United States involving the accidental ingestion of the poison by an infant when it was illegally used as an indoor rodenticide. TETS is not registered by the U. S. Environmental Protection Agency and its importation, manufacture and use in the U. S. are strictly forbidden [10]. Aside from its high toxicity, TETS's relatively simple manufacturing process and its remarkable stability in water render the neurotoxin a potentially persistent environmental contaminant [11,12].

Thus far, the focus of development of analytical methods has mainly resided in the use of LCMS- and GC-MS-based approaches to quantitate TETS in different matrices using various extraction protocols [13-16]. To this end, we have sought to investigate in detail the interactions between TETS and a panel of cyclodextrins (CyDs) using NMR spectroscopy. We anticipate that these initial spectroscopic studies would not only yield an understanding of the resulting host-guest interactions but more importantly,

they will provide insight into the feasibility of using cyclodextrins as scaffolds for TETS detection and sequestration.

Cyclodextrins are cyclic oligosaccharide structures composed of glucose units joined together via α1,4-glycosidic linkages (Figure 2a). The type of linkage that holds these units together gives rise to a well-defined, rigid, three-dimensional structure resembling that of a truncated cone (*i.e.* frustum) endowed with a hydrophobic interior and a hydrophilic exterior (Figure 2b) [17-20]. The number of glucose units directly influences CyD physical properties such as water solubility and cavity size. Due to their thermodynamic stability and ease of preparation at the industrial level, the three most commonly employed CyDs by most laboratories these days, the number of glucose residues composing these are 6, 7, and 8 corresponding to α-, β-, and γ-CyD respectively. Historically, cyclodextrins have been attractive molecular scaffolds as their propensity to form inclusion complexes is strictly governed by the size and hydrophobic landscape of the guest molecule. This type of host-guest association event has found a plethora of applications particularly in the pharmaceutical field where the bioavailability, solubility and stability of commonly used drugs have been enhanced as a result of their complexation with CyDs [21]. Classes of compounds that have historically exhibited strong binding affinities to cyclodextrins, most particularly with β-CyD, are ones possessing the adamantane geometry in their structure [22]. One such compound is adamantane carboxylic acid (Figure 2c), a molecule that possesses one of the highest complex stability constants known for β-CyD (K = 3.99 x $10^4 \pm 18$ M^{-1} in H_2O) [23,24], and is therefore commonly employed as a disruptor of β-CyD interactions with other small and large molecules [25,26]. Close examination of the atomic arrangement in TETS leads to the observation that its caged nature compellingly resembles that of the adamantane structure; this in conjunction to its compact, non-polar landscape prompted us to explore the feasibility of using CyDs as potential sequestering species for this neurotoxin.

74

75 The objective of this work was the investigation of inclusion complex formation between TETS and a
76 panel of CyDs that included native α, β, and γ-CyDs as well as the β-CyD chemically-modified
77 counterparts heptakis-(2,6-di-*O*-methyl)-β-CyD (DiOMe-β-CyD), heptakis-(2,3,6-tri-O-methyl)-β-CyD
78 (TriOMe-β-CyD) and heptakis-(2,3,6-tris-(2-hydroxypropyl))-β-CyD (2HP-β-CyD) and assess the
79 binding constants and complex stoichiometry by NMR. Furthermore, two-dimensional rotating-frame
80 Overhauser effect NMR spectroscopy (ROESY) has been employed to directly demonstrate the
81 proximity between the interior protons in β-CyD and 2HP-β-CyD (*i.e.* H_3 and H_5) and the methylene
82 protons in TETS.

2. Materials and methods

2.1 Chemicals and Reagents

TETS was synthesized according to a published procedure [27] and recrystallized from hot acetone to yield the product as pure, translucent cubic crystals in 60% yield. All cyclodextrins used in this study were purchased from TCI America (Portland, OR) and used as received. The heptakis-(tris-(2,3,6-tri-O-methyl))-β-CyD was synthesized according to a published procedure [28]. Deuterated solvents used ($CDCl_3$ and D_2O) for the NMR experiments were purchased from Sigma-Aldrich Co (St. Louis, MO).

2.2 NMR spectroscopy

All NMR spectra were acquired at 30.0 ± 0.1 ^{o}C using a Bruker Avance 600 MHz spectrometer (Bruker Biospin, Billerica, MA.) with a broadband inverse probe equipped with *z* gradients and a Bruker Cryoplatform. The NMR data were processed with the Bruker TopSpin 3.0 software. Solutions for analysis were prepared in 99.96% D_2O and the chemical shifts used for the Job plot derivatization were referenced to an internal standard of $CDCl_3$ fixed at 7.26 ppm. For each ^{1}H NMR experiment, 8 transients were collected into 65536 data points using a 1.5 s relaxation delay. Prior to the Fourier transformation the free induction decays (FIDs) were zero filled to 65536 points and apodized by multiplication with an exponential decay multiplication to 1.0 Hz line broadening. For the two-dimensional ROESY experiments on the 1:5 TETS:β-CyD and the 1:5 TETS:2HP-β-CyD complexes, the spectra were acquired using 2048 data points with 512 increments, 32 scans for each increment and a continuous wave spin lock with a 300 ms mixing time at 30.0 ± 0.1 °C. Data was collected in a phase sensitive fashion in addition to using a water suppression scheme aided with pulsed field gradients over 5410 Hz and 2860 Hz spectral windows for the 2HP-β-CyD and β-CyD, respectively.

3. Results and discussion

Several experimental protocols for the assembly of CyD inclusion complexes along with methods aimed to establish their correct geometry, in both the liquid and solid states, benefit from a vast amount of growing literature [22,29]. The first goal in our project was to assess the formation of inclusion complexes between TETS and the panel of six cyclodextrins. As previously mentioned, a key assumption in our hypothesis is the observation that the structural motif of TETS is amenable for complexation with a given CyD. Water was chosen as the solvent in our studies for two important reasons: the first one is based primarily on the favorable solubility exhibited by cyclodextrins in this medium. More importantly, a given community's water and food supplies would be greatly affected following an accidental environmental release of TETS. Thus, the data obtained from this type of study would be relevant and applicable to real case scenarios.

3. 1 Complex stoichiometry and binding constants determination

Our initial focus was to derive a Job plot using the continuous variation method that would correctly describe the stoichiometric nature of the inclusion complex formed (if any) between TETS and the cyclodextrins in our study [30]. Table 1 supplies the observed changes in chemical shift (δ) for the TETS methylene protons as a function of the TETS:CyD molar fraction (*r*) for all six CyDs described above. It is noteworthy to mention that during the course of our studies we were limited by the solubility of TETS for the preparation of the stock solutions, thus a 0.7 mM TETS solution was the most concentrate solution we could prepare and even in this instance full dissolution of the material was only achieved after mild heating followed by sonication. Furthermore, even though the solubility of the

cyclodextrins used in this study was found to be optimal in D_2O, the complete dissolution of β-CyD and its methylated analogs, DiOMe-β-CyD and TriOMe-β-CyD, to yield a 0.7 mM stock solution demanded gentle heating [31].

Regarding the contents of Table 1, we begin with the effect that changing the TETS:CyD molar fraction (*r*) has on the chemical shift of TETS when α-CyD is used. It is evident that there is little to no deviation observed for the original TETS chemical shift of 5.5814 ppm throughout the experiment as the TETS:α-CyD molar fraction (*r*) is systematically varied. Indeed, the measured Δδ values for $r = 0.2$, 0.4 and 0.6 were 0.0001 ppm, a practically negligible chemical shift change when considering the magnitude of TETS' signal linewidth (0.0003 ppm). This observation was interpreted as the absence of an inclusion complex between TETS and α-CyD at 30 ^{o}C suggesting that the cavity exhibited by α-CyD may be too small (5.7 Å diameter at the wide rim) to accommodate the TETS molecule [32] in an energetically favorable manner. Column 6 of Table 1 shows the change in chemical shift (Δδ) for TETS protons while varying the TETS:β-CyD molar fraction (*r*). The data reveal a noticeable difference from that obtained with α-CyD, as there is an initial, large downfield shift of the TETS signal from 5.5814 ppm to 5.6066 ppm (Δδ = 0.0252 ppm), and as the TETS:β-CyD molar fraction varies from $r = 0.2$ to $r = 0.8$ the signal shifts upfield to the final value of 5.5890 ppm corresponding to $r = 0.8$. This large chemical shift change experienced by TETS in the presence of β-CyD suggests some type of interaction (*i.e.* inclusion complex formation) between these two species. We postulate that as the outer rim in β-CyD (7.8 Å in diameter) is larger than that of α-CyD, it now offers a wide enough entrance to β-CyD's interior for TETS, thus resulting in favorable interactions within the cavity (van der Waals) to form a relatively long-lived inclusion complex observable by NMR. Next in Table 1 is the data involving the

chemical shift change in the TETS' protons while varying the TETS:γ-CyD molar fraction (*r*) (Column 8). The data reveal a subtle yet observable difference from those obtained with α-CyD. There is an initial downfield chemical shift from 5.5814 to 5.5834 ppm ($\Delta\delta = 0.0020$, for $r = 0.2$). Subsequent upfield shifts in small increments by the signal are observed leading to a final chemical shift value of 5.5821 ppm for $r = 0.8$. Thus, the data obtained from these set of experiments may be interpreted as depicting the formation of a weak inclusion complex between TETS and γ-CyD. The weaker nature of the association between TETS and γ-CyD may be attributed to the larger cavity size and outer rim diameter (9.5 Å) of this host. Even though in theory the large cavity of γ-CyD should allow for the entrance of the TETS molecule, such event does not benefit from further stabilizing hydrophobic interactions in the interior of the CyD to properly support an inclusion complex [33].

We now turn to the first chemically-modified CyD, TETS:2HP-β-CyD, and consider columns 9 and 10 in Table 1. These data reveal that the chemical shift modification due to the presence of the CyD is on the order of that observed for native β-CyD. As it can be observed in Table 1, there is an initial, large downfield shift of the TETS signal from 5.5814 ppm to 5.5987 ppm ($\Delta\delta = 0.0172$ ppm), and as the TETS:2HP-β-CyD molar fraction values vary from $r = 0.2$ to $r = 0.8$ the signal begins shift upfield to the final value of 5.5851 ppm corresponding to $r = 0.8$. The large chemical shift change in TETS once again could be interpreted as partial evidence for the formation of an inclusion complex. The actual diameter of the wide rim in 2HP-β-CyD is difficult to accurately evaluate as a locked set of hydroxyl groups on it (C2 and C3 hydroxyl groups as in the native β-CyD) no longer exists, but three-carbon long sidechains possessing more degrees of freedom and thus more attainable conformations. Nevertheless, the data indicates that this modified analog of β-CyD still possesses the ability to complex TETS. The

fifth and sixth sets of data shown in Table 1 (Columns 11-14) describe the chemical shift changes of TETS with the two methylated β-CyD analogs. Before discussing the data for the last set of two CyDs used in this study, the rationale behind choosing these as part of our testing panel warrants a separate discussion. An important part of our initial hypothesis aimed to explain the strong interactions between TETS and native β-CyD was that such inclusion complex could not be only benefiting rom the van der Waals forces between TETS and β-CyD's interior. Therefore, we reasoned that the binding event could be experiencing significant augmentation from hydrogen bonding interactions, possibly between the sulfamide oxygen in TETS and the C2/C3 hydroxyl groups in β-CyD's wide rim. Therefore, we reasoned that if we were to systematically disrupt these interactions (*e.g.* via methylation of one or both of the C2 and C3 hydroxyl groups) we should observe a decrease in the complex's stability constant (*vide infra*). To this end, we included the partially methylated β-CyD analog, 2,6-DiOMe-β-CyD, in which one of the hydroxyl groups (C2) in the rim is methylated. In addition, we felt that it was necessary to include the β-CyD analog that is methylated at both C2 and C3 positions, and to achieve this we synthesized 2,3,6-TriOMe-β-CyD. In this latter case, all the hydroxyl groups including those in C2 and C3 have been capped with methyl groups. As expected the initial, downfield shifts for both methylated CyDs (columns 12 and 14) are significantly less than that of their native β-CyD and 2HP counterparts. For the dimethylated CyD (2,6-DiOMe-β-CyD) the initial value of $\Delta\delta$ ($r = 0.2$, *i.e.,* largest CyD mole fraction) is 0.0062, an order of magnitude lower than that for native β-CyD. Capping all hydroxyl groups on the large rim with methyl groups lowers this value even further ($\Delta\delta = 0.0048$ at $r = 0.2$). Overall, as the TETS:2,6-DiOMe-β-CyD molar ratio varies from $r = 0.2$ to $r = 0.8$, the signal experiences an upfield chemical shift to a final value of 5.5833 ppm at $r = 0.8$. For the TriOMe-β-CyD complex, we observe a upfield chemical shift ranging from 0.0048 to 0.0018 until the final value of 5.5832 ppm for $r = 0.8$. As a final comment, although we have previously invoked maximum $\Delta\delta$ values

193 as corollaries to binding strength, only the direct calculation of the binding constants from titration
194 experiments can be considered a proper quantification of the complex binding affinity.

195

196 The data described in Table 1 can be plotted to give the parabolic traces (Job plots) shown in Figure 3.
197 These traces yield the stoichiometric nature of the inclusion whether these are strongly or weakly
198 favored. In all cases, the Job plots are characterized by possessing a maxima centered at $r \sim 0.5$,
199 strongly suggesting that the inclusion complexes between TETS and the six CyDs used in this study
200 favor a 1:1 stoichiometry.

201

202 *3.2 Determination of binding constants by NMR titration*

203 Armed with these initial insights, we pursued the determination of the binding constant K between
204 TETS and the CyDs studied herein by making use of a well established NMR titration protocol. This
205 approach has found tremendous success in the determination of binding constants for inclusion
206 complexes between a variety of organic compounds and CyDs [34-37]. NMR titration experiments
207 were not conducted for the TETS:α-CyD case since no observable changes in the TETS' protons
208 chemical shift upon incubation with the CyD was identifiable. Therefore, we focused our efforts on
209 determining the binding constants for the other remaining five CyDs. Solutions were made at a 0.24 mg
210 mL^{-1} concentration of TETS in D_2O, gradually titrated with a 50.0 mg mL^{-1} solution of the given CyD,
211 and the NMR spectra immediately taken after each addition. Titration experiments were performed in
212 duplicates and yielded the curves shown in Figure 4. Non-linear regression analysis of the data points
213 derived from the curves yielded a $K = 537 \pm 26$ M^{-1} for the TETS:β-CyD inclusion complex and a $K =$
214 514 ± 49 M^{-1} for the TETS:2HP-β-CyD complex. The next binding constant in magnitude was the one

corresponding to the TETS:2,3,6-TriOMe-β-CyD complex ($K = 123 \pm 55$ M^{-1}). Lastly, the lowest binding constants measured corresponded to the TETS:γ-CyD ($K = 90 \pm 29$ M^{-1}) and the TETS:2,6-DiOMe-β-CyD ($K = 89 \pm 45$ M^{-1}) complexes. These data are summarized in Table 2, which additionally includes both Log K data and the binding constant for TETS/1-adamantane carboxylic acid for comparison.

3.3 Two-dimensional ROESY experiments

After determining that β-CyD and 2HP-β-CyD formed the strongest inclusion complexes with TETS we turned to using two-dimensional rotating frame Overhauser effect (2D-ROESY) NMR spectroscopy. The 2D-ROESY experiments were conducted with the goal of obtaining information on the structural features akin to each one of the inclusion complexes and its usefulness lies in the real-time, dynamic measurement of through-space interactions between protons present in the guest molecule (*i.e.* TETS) and the interior of a host (*i.e.* β-CyD or 2HP-β-CyD). In our system of interest, we are interested with the interactions between TETS's methylene protons and the H_3 and H_5 protons that decorate the interior of the CyD cavity which are suitably positioned to interact with the guest molecule's protons (Figure 5).

The full 2D-ROESY spectrum of the TETS:β-CyD system is shown in Figure 6. A brief examination of the spectrum shows that the methylene protons of TETS show well-defined cross-peaks to the H_3 and H_5 protons in the interior of β-CyD, demonstrating that the neurotoxin, when complexed, lies in close proximity to the cavity of the macrocycle. The observation that a more intense cross-peak is observed for TETS: β-CyD-H_3 than for TETS: β-CyD-H_5 strongly suggests that the time-averaged distance in the former case is smaller than in the latter scenario. Considering the geometry of the CyD molecule (large

237 and small outer rims) and the moderate ROESY mixing times used for the experiment these results
238 support the notion that whether or not a true inclusion complex is formed, the positioning of the TETS
239 molecule seems to lie in close proximity to the larger, outer rim of the macrocycle.

240

241 Shown in Figure 7 is the 2D-ROESY spectrum of the TETS:2HP-β-CyD system. In addition to the
242 expected intramolecular cross-peaks, intermolecular cross-peaks are also apparent. In particular, cross
243 peaks between H_3, H_3', H_5, and H_5' of 2HP-β-CyD and the TETS protons can be observed (Fig. 7).
244 Again, these provide direct evidence of an inclusion complex due to interactions between the inner
245 protons of 2HP-β-CyD and TETS, regardless of the strength of this interaction [38-40]. Both ROESY
246 experiments performed herein can only be used as a demonstration of host-guest spatial proximity (*i.e.*
247 inclusion complex formation). Although the extraction of quantitative information about time-averaged
248 distances between interacting protons is possible, this is not possible within our experimental setup.
249 Nevertheless, 2D-ROESY remains a powerful tool in NMR spectroscopy for establishing intermolecular
250 interactions within the 1-10 Å range.

251

252 **4. Conclusion**

253 The binding constants (*K*) for the inclusion complexes formed between TETS and a panel of CyDs were
254 determined using NMR spectroscopy. All CyDs evaluated in this study were observed to form inclusion
255 complexes of varying degrees of magnitudes with TETS (all favoring 1:1 stoichiometric ratios) with the
256 exception of α-CyD, which did not appear to form a complex with the neurotoxin. TETS was found to
257 bind most strongly to native β-CyD ($K = 537 \pm 26$ M^{-1}) and its chemically modified counterpart 2HP-β-
258 CyD ($K = 514 \pm 49$ M^{-1}). 2D-ROESY NMR was found to be instrumental in the direct observation of

259 inclusion complexes formation. The measured binding constants described herein will guide us in the
260 design of technologies based on cyclodextrin platforms targeted for the capture of TETS. Current
261 efforts at the Forensic Science Center (FSC) include the synthesis of CyD-modified organic/inorganic
262 matrices for use in remediation technologies as well as the synthesis of novel chemically modified CyDs
263 that feature enhanced binding affinities for the neurotoxin.

264

265 **Safety**

266 Tetramethylenedisulfotetramine is an extremely hazardous chemical (human oral LD_{50} = 0.1 mg kg^{-1})
267 and is a persistent environmental contaminant. Proper personal protective equipment (PPE) should be
268 worn at all times when handling even small amounts of it. All solid and liquid waste containing TETS
269 should be treated as extremely hazardous and extreme caution should be employed in its disposal.

270

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274 AC52-07NA27344.

FIGURE CAPTIONS

Figure 1. Chemical structure of TETS.

Figure 2. (a) Chemical structures of the three most common CyDs; (b) a three-dimensional representation of β-CyD showing the conical frustum nature shared by this family of macrocycles; and (c) the structure of adamantane carboxylic acid, one of the strongest binders known to β-CyD.

Table 1. TETS chemical shift and chemical shift changes ($\Delta\delta$) as a function of TETS:CyD molar ratio (*r*) for the six CyDs at 30°C. [a]The $\Delta\delta$ given is defined as the difference in the TETS chemical shift in the presence of CyD from that of TETS in D_2O alone ($\Delta\delta = \delta - \delta_w$). TETS peak linewidth on the order of 0.2 Hz making the uncertainty in the measurement ca. ±0.00017 ppm.

Figure 3. Continuous variation Job plots derived from the chemical shift change in TETS as a function of TETS:CyD molar fraction (*r*). Parabolic fits to the data (forced through the origin) are merely supplied as visual guides.

Figure 4. a) ^{1}H NMR titration experiments demonstrating the change in chemical shift ($\Delta\delta$) in TETS's methylene protons as a function of increasing CyD concentration at 30°C. Black lines represent the nonlinear regression-derived fits to these data from which binding constants were extracted. b) Bar graph representation showing the relative magnitudes of binding constants among the CyDs used in this study.

Figure 5. Three dimensional diagram showing the glucosyl H_3 and H_5 protons (left) decorating the interior of all CyDs (β-CyD shown). A diagram of a hypothetical TETS:CyD complex demonstrating the strategic positioning of these interior protons (right) as invaluable key reporting species in 2D-ROESY NMR experiments.

Figure 6. 2D-ROESY (HOD suppression) NMR spectrum of the 1:1 TETS:βCyD inclusion complex at 30 ^{o}C. Cross-peaks showing the interactions between TETS and the H_3 and H_5 protons of β-CyD are indicated by the red arrows.

Figure 7. 2D-ROESY (HOD suppression) NMR spectrum of the 1:1 TETS:2HP-β̃CyD inclusion complex at 30 ^{o}C. Cross-peaks showing the interactions between TETS and the H_3, H_3', H_5 and H_5' protons lining the interior of 2HP-β-CyD are indicated by the red arrows.

Table 1. TETS chemical shift and chemical shift changes (Δδ) as a function of TETS:CyD molar ratio (*r*) for the six CyDs at 30°C. [a]The Δδ given is defined as the difference in the TETS chemical shift in the presence of CyD from that of TETS in D_2O alone ($\Delta\delta = \delta - \delta_w$). TETS peak linewidth on the order of 0.2 Hz making the uncertainty in the measurement ca. ±0.00017 ppm.

	D_2O	α-CyD		β-CyD		γ-CyD		2HP-β-CyD		2,6-DiOMe-β-CyD		2,3,6-TriOMe-β-CyD	
TETS:CyD (*r*)	δ_w	δ	Δδ[a]	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ
0.2	5.5814	5.5815	0.0001	5.6066	0.0252	5.5834	0.0020	5.5987	0.0172	5.5876	0.0062	5.5862	0.0048
0.3	5.5814	5.5818	0.0004	5.6037	0.0224	5.5832	0.0018	5.5972	0.0158	5.5867	0.0053	5.5859	0.0045
0.4	5.5814	5.5815	0.0001	5.6013	0.0200	5.5831	0.0017	5.5940	0.0126	5.5862	0.0048	5.5851	0.0037
0.5	5.5814	5.5819	0.0005	5.5985	0.0171	5.5828	0.0014	5.5924	0.0110	5.5854	0.0040	5.5847	0.0033
0.6	5.5814	5.5815	0.0001	5.5951	0.0137	5.5825	0.0011	5.5891	0.0077	5.5849	0.0035	5.5841	0.0027
0.7	5.5814	5.5823	0.0009	5.5924	0.0110	5.5823	0.0009	5.5878	0.0064	5.5842	0.0028	5.5846	0.0032
0.8	5.5814	5.5816	0.0002	5.5890	0.0076	5.5821	0.0007	5.5851	0.0037	5.5833	0.0019	5.5832	0.0018

Table 2. Binding constants K (M^{-1}) and Log_{10} K values for all CyD:TETS complexes studied by NMR titration experiments. Shows for comparison is the data for the complex formed between β-CyD and 1-adamantane carboxylic acid (AdaCOOH) (data from ref. [23]). Error shown in parentheses.

Host Cyclodextrin	***K*** **(M^{-1})**	**Log_{10} *K***
β-CyD	538 (26)	2.73
γ-CyD	86 (5)	1.93
2HP-β-CyD	514 (49)	2.71
2,6-DiOMe-β-CyD	91 (28)	1.96
2,3,6-TriOMe-β-CyD	123 (5)	2.09
β-CyD:AdaCOOH*	3950 (150)	3.60

FIGURES

Figure 1.

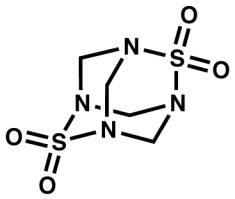


Figure 2.

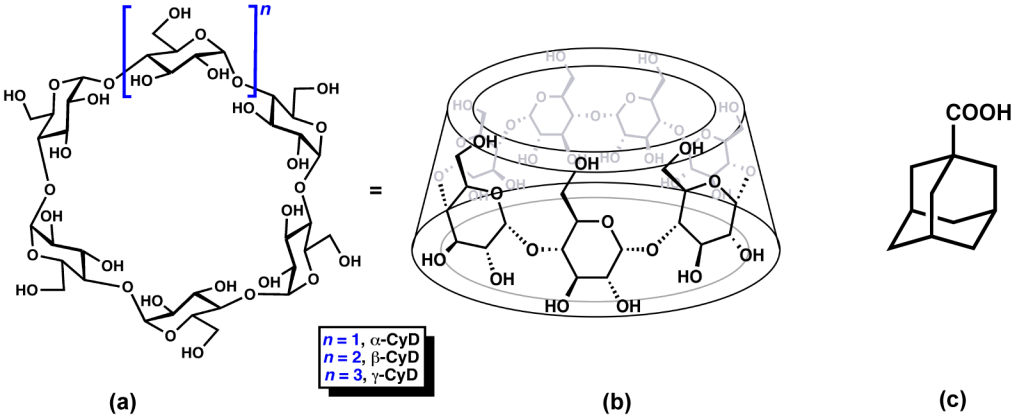
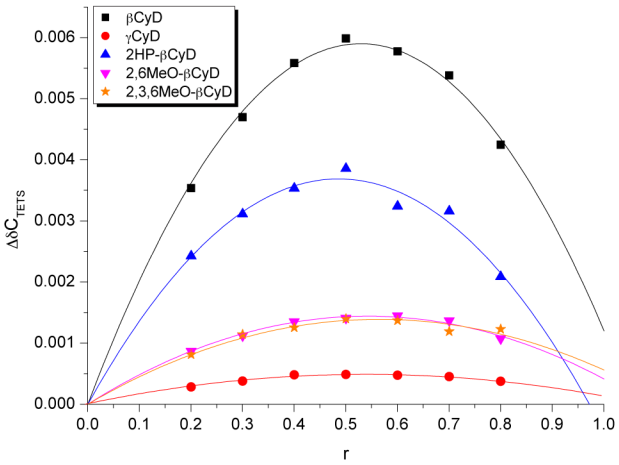


Figure 3.


βCyD
γCyD
2HP-βCyD
2,6MeO-βCyD
2,3,6MeO-βCyD
ΔδC_TETS
r
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.0
0.1
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0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Figure 4.

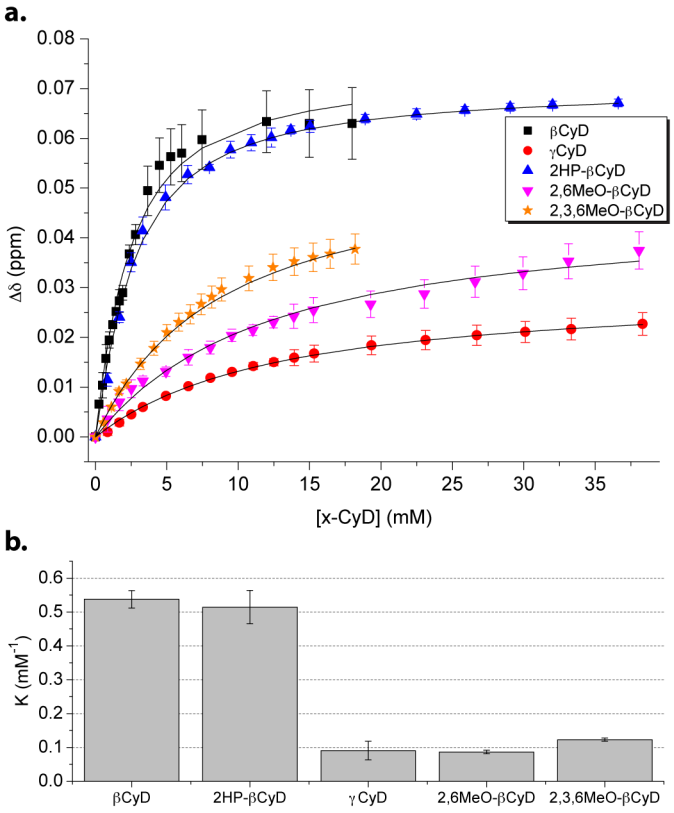

a.
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Δδ (ppm)
0
5
10
15
20
25
30
35
[x-CyD] (mM)
βCyD
γCyD
2HP-βCyD
2,6MeO-βCyD
2,3,6MeO-βCyD
b.
0.6
0.5
0.4
0.3
0.2
0.1
0.0
K (mM-1)
βCyD
2HP-βCyD
γCyD
2,6MeO-βCyD
2,3,6MeO-βCyD

Figure 5.

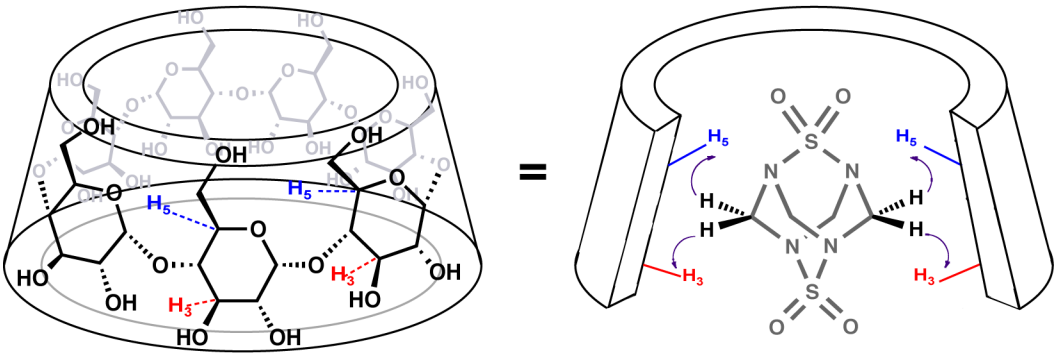


Figure 6.

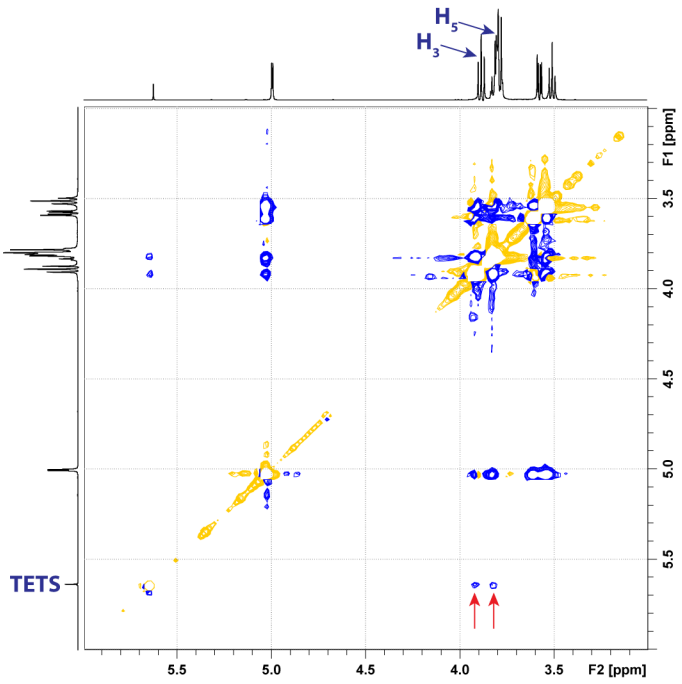
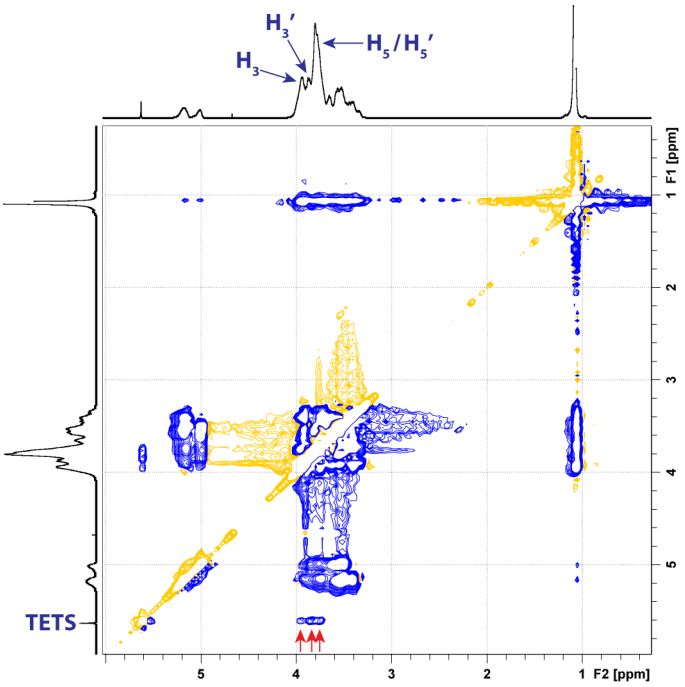


Figure 7.



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